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SPECIALIZATION, HYBRIDIZATION, AND MUTATION IN THE CEREAL RUSTS*

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INTRODUCTION

The small-grain cereals, wheat, oats, barley and rye, are among man's most important food-producing plants. As the several rusts to which they are subject are the most serious diseases of these plants and perhaps the greatest hazards in their production, it is but natural that they should have received more scientific attention than the rusts of any other group of plants. Intensive study of these rusts during the last half century has yielded a great and steadily growing fund of knowledge concerning them. It is the purpose of this paper to review, as briefly as is consistent with clarity, the main contributions to our knowledge that have accrued from two important discoveries—that of physiologic specialization, and the discovery of the existence of heterothallism in the rusts. The former showed these rusts to be made up of numerous parasitically different strains; the latter opened the way to studies on hybridization between these strains, which in turn have thrown considerable light on their genetical constitution.

POLYMORPHISM AND HETEROECISM

Although rusts have affected cereals from prehistoric times to the present, scientific study of these important pathogens goes back only about 200 years. Hooke pictured the teliospores of a rust in his "Micrographia", published in 1665; and Micheli made a beginning at rust nomenclature when, in his "Nova Plantarum Genera", published in 1729, he gave the name *Puccinia* to a rust that later was transferred to the genus *Gymnosporangium* (4). Classification of the rusts, however, did not really begin until about the end

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of the eighteenth century when Persoon (164, 165) recognized the rusts as a distinct group of fungi and divided those known to him into several genera.

During the first half of the nineteenth century much work was done on the taxonomy of the rusts, despite the fact that investigators, under the influence of the theory of spontaneous generation of lower organisms, generally did not accept rusts as living and self-propagating species of plants. Most of this work was later to undergo modification because the creators of new genera and species did not understand the life cycles of the rusts and consequently classified different spore forms that belonged to the life cycle of the same rust as different genera. The realization that a single rust might possess more than one spore form was brought about chiefly by the epochal work of the Tulasne brothers and Anton de Bary. The Tulasnes accepted the common association of urediospores and teliospores as evidence of their common origin (222, 223) and described the production of the sporidia by the promycelia of germinating teliospores (221). They showed, in other words, that some rusts, at least, were polymorphic. The fact that some species of rust spend a part of their life cycle on one host species and part on another was demonstrated by de Bary (10) who infected barberry with sporidia from teliospores of *Puccinia graminis* with the result that aecia of the species known as *Aecidium Berberidis* were produced. This experiment showed that the two previously accepted species of rust merely represented different parts of the life cycle of one rust. He thereby established the fact of "heteroecism", a name that he coined to describe the alternation of hosts in the life cycles of certain rusts. This discovery was followed by numerous demonstrations of rust species that produce pycnia and aecia on one species of host plant and uredia and telia on another (108).

SPECIALIZATION OF CEREAL RUSTS

Historical review

Species of rusts, as of other plants, are based on morphological characteristics. The fact that there exist within some morphological species well defined strains that can be differentiated from one another by physiological criteria (*i.e.*, the specialized ability to attack only certain hosts) was demonstrated in 1894 by Eriksson and Henning (46) and Eriksson (41) for *Puccinia graminis* Pers.

They showed that in Sweden there were several of these specialized strains and designated them as *formae speciales* (*forma specialis,* sing.). About the same time Hitchcock and Carleton (87) found in infection experiments conducted in Kansas that "both wheat and oats are easily infected by rust from the same kind of grain, but not by the same kind of rust from other grains", and they concluded that "the rusts of various cereals are probably physiological species". In the present paper these *formae speciales* or physiological species will be referred to as "varieties" of the rust.

In *P. graminis* specialization is not necessarily identical, though it appears to be rather similar, in different parts of the world. Eriksson (41, 42, 44) and Eriksson and Henning (47), as a result of experiments with European rust collections, divided the rust into the following varieties: *a*) *Secalis* (on rye, barley and certain grasses); *b*) *Avenae* (on oats and certain grasses); *c*) *Tritici* (on wheat); *d*) *Airae* (on *Aira caespitosa*); *e*) *Agrostidis*[1] (on *Agrostis* spp.); *f*) *Poae* (on *Poa* spp.); to which Eriksson (45) added a seventh variety, *Epigaei,* on *Calamagrostis* spp. In North America (189, 193, 204) the varieties *Tritici, Secalis, Avenae, Agrostidis* and *Poae* have been found as well as the variety *Phlei-pratensis* which also occurs in Europe but was regarded by Eriksson as a separate species. In the United States there has recently been collected (49) on *Agropyron spicatum* what appears to be a new variety of *P. graminis,* differing pathogenically from those mentioned above. In Japan *Puccinia culmicola* Diet., with its aecia (*Aecidium Berberidis-Thunbergii* P. Henn.[2]) on *Berberis Thunbergii,* has been regarded as a variety of *Puccinia graminis,* owing to its ability to attack *Agropyron* and rye (6).

The differences between these varieties of *Puccinia graminis* are not solely physiological, for it has been demonstrated (47, 114, 191, 193, 204, 235) that they differ somewhat in the size of their spores, particularly the urediospores. The morphological differences, however, are not great enough to enable each variety to be identified

[1] Some writers quoted in this paper have used the ending *-is* for specific names based on *Agrostis* and *Calamagrostis* rather than that of *-idis* originally used by Eriksson (41). For the sake of uniformity the latter ending is used exclusively in this paper.

[2] Moreover, the Sydows (Monographia Uredinearum Vol. 4: 245. 1923) give the aeciospore wall as 1 μ thick to 2–6 μ at the apex, a feature characteristic of the aeciospores of *Puccinia graminis* on *Berberis vulgaris.* They comment: "Diese Art steht dem *Aec. Berberidis* Gmel. äusserst nahe und ist wohl damit identisch, da morphologische Unterscheide kaum wahrnehmbar sind".

except perhaps by means of statistical studies of the measurements of many spores.

Puccinia triticina Erikss. (*P. rubigo-vera Tritici* (Erikss. & Henn.) Carl.) is one of a group of gramineous leaf rusts belonging to the old species *P. rubigo-vera* (DC.) Wint. which Eriksson and Henning (46) in 1894 divided into the two species *Puccinia glumarum* Erikss. & Henn. and *Puccinia dispersa* Erikss. & Henn. The latter species Eriksson (41, 42) resolved into four varieties: *a*) *Secalis* (on rye); *b*) *Tritici* (on wheat); *c*) *Bromi* (on *Bromus* spp.); and *d*) *Agropyri* (on *Agropyron repens*). He later (43) revised this nomenclature by raising the variety *Tritici* to the status of a species (*P. triticina* Erikss.) as he did also the variety *Secalis* to which he gave the name *P. dispersa* Erikss. These two species are morphologically similar, but *P. triticina* produces aecia on *Thalictrum* spp. (93, 123) and, in Siberia, on *Leptopyrum* (*Isopyrum*) *fumarioides* L. (20), whereas *P. dispersa* forms aecia on *Anchusa* spp. (11, 124) and is also reported to attack *Echium tuberculatum* (156). As they are but two among many rather similar rusts with uredia and telia on gramineous hosts and aecia on the Ranunculaceae or Boraginaceae, some taxonomists (5, 34, 125) prefer to regard them as merely varieties of a complex species, as, indeed, Eriksson did originally. To this group of grass rusts Arthur (5), following Mains (123), has given the name *Puccinia rubigo-vera* (DC.) Wint., and Cunningham (34), the name *Puccinia Elymi* Westnd. In the present paper, however, the names *P. triticina* and *P. dispersa* are retained for the two cereal rusts, as this has been the custom in most plant pathological literature.

Puccinia glumarum (Schm.) Erikss. & Henn. is the only one of the cereal rusts for which no alternate host is known (77, 211, 218). As stated above, this rust was separated from *P. rubigo-vera* by Eriksson and Henning. It was divided by Eriksson (41) into five varieties: *a*) *Tritici* (on wheat); *b*) *Secalis* (on rye); *c*) *Elymi* (on *Elymus arenarius*); *d*) *Agropyri* (on *Agropyron repens*); and *e*) *Hordei* (on barley). Later investigations (70, 79, 145, 207, 208, 210, 211, 219) have shown that specialization in this rust is less clear-cut and definite than Eriksson supposed; and the existence of his varieties has been questioned. Nevertheless it has been shown (48, 210, 211) that certain strains of the rust have a preference for wheat, others for barley or for rye; and that specific strains occur on *Agropyron repens* and *Hordeum murinum*.

The specialization of *Puccinia coronata* Cda. is among the most complex to be found in the grass rusts. De Bary (11) first showed the ability of this rust to produce aecia on *Rhamnus*. Klebahn (107, 108) divided the rust into two species: *P. coronata* Kleb., with aecia on *Rhamnus Frangula;* and *P. coronifera* Kleb., with aecia on *R. cathartica*. More recent work (19, 35, 136, 209, 219) has failed to substantiate this division; and *Puccinia coronata* Cda. is now generally regarded as a species made up of several varieties, of which some produce aecia on different species of *Rhamnus,* while others form aecia on *Lepargyraea* or *Elaeagnus*. Cunningham (34) recognizes the six varieties *Agrostidis, Avenae, Calamagrostidis, Glyceriae, Holci* and *Melicae;* and Arthur (5) admits the additional varieties *Agropyri, Alopecuri, Festucae, Lolii* and *Phalaridis*. Brown (18) reports the occurrence in England of yet another variety, *Arrhenatheri*. Fraser and Ledingham (51) found the following varieties to occur in the Prairie Provinces of Canada: *Avenae* (aecia on *R. cathartica,* uredia and telia on *A. sativa* and *A. fatua*); *Calamagrostidis* (aecia on *R. alnifolia,* uredia and telia on *Calamagrostis* spp. and *Scolochloa festucacea*); *Bromi* (aecia on *Lepargyraea canadensis,* uredia and telia on *Bromus ciliatus, B. latiglumis* and *B. Porteri*); *Elaeagni* (aecia on *Elaeagnus commutata,* uredia and telia on *Calamagrostis elongata*).

In *P. coronata* there is some evidence that the pathogenic characteristics of at least some of the varieties may be changed by passage through the alternate host. Dietz (35) states that urediospores of *P. coronata Avenae* cause only subnormal infection on *Holcus lanatus;* yet aeciospores produced by the *Avenae* variety of the rust on several species of *Rhamnus* were able to rust this grass heavily. Similarly aeciospores of the variety *Calamagrostidis* showed greater ability to rust oats than did urediospores of that variety; and conversely aeciospores of the *Avenae* variety rusted *Calamagrostis canadensis* which *Avenae* urediospores normally do not. These results led Brown (19) to study the effect of passage through the alternate host on the pathogenicity of the varieties *Calamogrostidis, Holci* and *Alopecuri*. Her experiments, however, indicated "that the varieties of *Puccinia coronata* are not altered appreciably by their passage through the aecidial host". She found, furthermore, that these varieties did not readily intercross with each other.

Puccinia anomala Rostr. (*Puccinia simplex* Erikss. & Henn.)

with aecia on *Ornithogalum* (124, 217) is considered by most authorities to be confined in its uredial and telial stages to cultivated barley. There are, however, reports of its ability to attack certain wild species of *Hordeum* (79, 234), and d'Oliveira (154) has collected on *H. murinum, H. maritima* and *H. pratense* strains that parasitized these grasses but were unable to attack cultivated barley. These strains he regards as highly specialized races or varieties of *P. anomala.* He, however, did not demonstrate their ability to infect *Ornithogalum,* and some authorities might be inclined to look upon them as strains of *Puccinia Hordei* Fckl. (213).

From the time of Eriksson's discovery of specialization in *Puccinia graminis* in 1894 until 1917, the *formae speciales* or varieties described by him were regarded as the ultimate units of specialization. Realization that a further subdivision of these units could be made resulted from the discovery by Stakman and Piemeisel (203) of a strain of wheat stem-rust to which they originally gave the name *Puccinia graminis Tritici-compacti.* This strain differed from other wheat stem-rust that they had studied previously in that it produced weak infections on the hard spring wheats and the hard winter wheats (113, 204). When still other strains were found (118, 135, 141, 198) that could be differentiated by the reaction of wheat varieties, it became evident that the variety *Tritici* was specialized into physiologically or pathogenically different strains that could be distinguished by the reaction to them of varieties within the genus *Triticum* in much the same way as Eriksson's *formae speciales* could be distinguished by the reactions of genera or species of the family Gramineae. The economic significance of these discoveries stimuated the search for new "biologic forms", as they at first were called, with the result that 37 of them were described within five years (192). The term "biologic form" was later superseded by that of "physiologic form", which term eventually gave place to "physiologic race", the designation used in the present paper.

The study of intra-varietal specialization was soon extended to various other cereal rusts, with the result that every one of them is now known to be composed of a number of physiologic races.

Identification of physiologic races

An understanding of what is meant by physiologic races requires discussion of some of the factors involved in their identification.

As indicated above, physiologic races differ from each other pathogenically, and, as a physiological basis must be postulated for such differences, the term "physiologic race" seems appropriate. Even if all the hosts of a rust were physiologically identical, it would still be possible to identify a few physiologic races because pathogenically different strains of a rust produce different symptoms. If the host is entirely congenial to the rust, large uredial sori (uredia) are produced with little if any sign of chlorosis. If the host is not entirely congenial, the sori are smaller and an area of sharp chlorosis usually surrounds the uredial pustules. On an uncongenial host the rust will produce small uredia surrounded by necrotic areas. The fact that there is much physiological variation in the host as well as in the rust, makes it possible to identify numerous physiologic races. The various symptoms produced by a rust are known as "infection types" and are represented by symbols; and the different host varieties that have been found most suitable for displaying the diversity of infection types are referred to as "differential hosts" or "differential varieties". Infection types for use in the identification of physiologic races of *P. graminis Tritici* were described by Stakman and Levine, and these have been adopted generally for the other cereal rusts with whatever modifications are necessary to suit the individual characteristics of a rust. A description of the infection types, as applicable to stem rust of wheat, is given below:

0—Host immune
No uredia developed; hypersensitive flecks usually present, but sometimes there is absolutely no trace of mycelial invasion in the host tissues.

1—Host very resistant
Uredia minute and isolated; surrounded by sharp, continuous, hypersensitive necrotic areas.

2—Host moderately resistant
Uredia isolated and small to medium in size; hypersensitive areas present in the form of necrotic halos or circles; pustules often in green but slightly chlorotic islands.

3—Host moderately susceptible
Uredia medium in size; coalescence infrequent; development of rust somewhat subnormal; true hypersensitiveness absent; chlorotic areas, however, may be present.

4—Host very susceptible
Uredia large, numerous and confluent; true hypersensitiveness entirely absent, but chlorosis may be present when cultural conditions are unfavorable.

X—Host reaction heterogeneous
Uredia very variable, apparently including all types and degrees of infection on the same blade; no mechanical separation possible; on reinoculation small uredia may produce large ones and *vice versa*. Infection ill defined.

The first problem facing the original investigator of specialization in a cereal rust was that of discovering the particular host varieties most suitable for distinguishing physiologic races—a problem that usually required the testing of hundreds of varieties of the host. The differential varieties thus discovered differed, of course, for different rusts, and in some cases more than one group of differential varieties have been devised for the same rust.

It was soon found that a good differential host must possess certain characteristics if it is to be satisfactory for the distinguishing of physiologic races. Perhaps two of the most important characteristics are homozygosity, and stability of reaction to rust under fluctuating environmental conditions. The first can be obtained rather readily (except in rye varieties) by selection; the second can be secured only by rigidly discarding varieties whose reaction responds too readily to environmental influences.

Physiologic specialization in Puccinia graminis Tritici

The discovery of the presence of physiologic races in the *Tritici* variety of stem rust has already been discussed. As this is one of the most widely distributed of the cereal rusts and second to none in economic importance, it is natural that its specialization should have been studied intensively. In the United States and in Canada, surveys to determine the prevalence of physiologic races have been conducted annually from 1919 to the present time; and in Australia data are available for the period 1922 to 1938. In several other regions—South Africa (229), British East Africa (Kenya) (111, 112, 128, 129), India (133), the Argentine (225, 226)—surveys have also been carried out for periods of years. As a result of these studies and others too numerous to mention here, at least 200 physiologic races of this rust have been identified. Not all of these were collected from uredia or aecia gathered in nature. A considerable number were produced in experimental hybridization studies, and more than 50 of the races so produced have not yet been found to occur naturally.

As far as it is possible to judge of the pathogenic characteristics of the races present in different regions from comparisons of their infection types on the 12 wheat varieties used as differential hosts, it would appear that there are rather pronounced pathogenic differences between those occurring in widely separated regions. The

two emmer wheats, Vernal and Khapli, are resistant to all the physiologic races that have been common in North America since surveys of races were undertaken, the latter variety being resistant to all the races found in North America. In other regions, however, certain races attack these varieties severely. In South America (the Argentine, Brazil, Uruguay) race 42, which attacks Khapli heavily, at least in the seedling stage, is common (225, 226), and race 189, which attacks both Vernal and Khapli, has been found in Peru (56). In Abyssinia five races that attack Khapli have been described (188), two of them rather closely resembling race 42 which was originally discovered in Egypt as was also race 41 which likewise attacks Khapli (56). In India (133) race 42 is more common than any other race, and the two races next in order of prevalence attack the variety Vernal heavily. In Europe race 40, which attacks Vernal heavily and is extremely rare in North America, is one of the most commonly collected races (55, 81, 157). Examples of pathogenic differences in the rust need not, however, be confined to widely separated continents. It has been shown (205) that races which attack the variety Marquis heavily are very rare in southern Mexico but occur commonly in the northern part of the country—a fact indicating a very small amount of interchange of inoculum between the two regions.

The question of whether the predominant races of a region maintain their predominance year after year or periodically give way to other races, is of particular interest to investigators concerned with the development of new varieties because any such radical change is equivalent to a change in the pathogenicity of the rust. Evidence bearing on this question is available for at least two regions, North America and Australia, in which surveys for physiologic races have been conducted for many years. In North America, where data are available for a quarter of a century, several fluctuations have occurred in the relative abundance of particular races. During the period 1919 to about 1924, the two rather similar races 17 and 29 were more common than any other races (115, 142). From that time on they diminished in prevalence until during the early thirties they made up an inconsiderable part of the total isolates of races. In 1940 and 1941 they again increased to such an extent that they almost regained their earlier predominance (202). About 1925 a group of three similar races, 3, 18 and 36, assumed

a preponderance (142, 199, 232) that was maintained until shortly after 1930, since when they have been collected but rarely. In 1934 race 56, which was first collected in 1928 (163, 202), became rather suddenly the predominant race throughout the United States and Canada, a position it has maintained up to the present time. The virtual disappearance of races 3, 18 and 36 is paralleled by that of races 21 and 49. Race 21 was collected commonly each year until about 1940, since when its occurrence has become a rarity; race 49 was one of the common races from 1927 to 1933 but has occurred only sporadically in recent years.

In Australia, from which records are available for the years 1922 to 1938 (239), there have been somewhat comparable though less striking fluctuations in the prevalence of particular races. From 1922 to 1928, races 43, 44, 45 and 46 were present in considerable abundance in most years. Since then races 44 and 46 have not been collected, and the other two have occurred only sporadically. On the other hand, race 34, collected the first time in 1926, became the most prevalent race two years later and has since shown a large predominance over other races.

Only occasionally has it been possible to give a satisfactory reason for the origin of new races or their rise to prominence. Waterhouse (237, 239) has furnished reasons for supposing that race 11, which occurred naturally for the first time in Australia in 1935, had its origin in aecia derived from a natural selfing of race 34, the predominant Australian race. In 1928, seven years before the discovery of race 11 in the field (233), he isolated that race and race 56 from aecia that arose most probably from a selfing of race 34 or possibly from a cross of that race with race 43. His conclusion that race 11 probably arose from a selfing of race 34 is supported by unpublished work by the present writers in which the selfing of a pure culture of race 34 produced uredial cultures of race 11 as well as race 34.

It has been suggested (190) that the origin of race 56 in America is possibly also connected with aecia on barberry. This race was first found in nature in Iowa, Kansas and Nebraska in 1928, some of the collections being obtained from uredia in close proximity to barberry bushes (163). It was not collected in the uredial stage in Canada until 1931; but it was first identified in September, 1927, from aecia on an artificially infected barberry turned over to the

writers by J. H. Craigie (151). The occurrence of this race in both Canada and Australia in cultures derived from aecia is suggestive that its first appearance in the field may be attributed to natural infection from barberry. In regions where barberry is present, it is indeed likely that this shrub plays the chief part in the origin of new races.

Why one race rather than another should multiply and gain prominence is, in most cases, equally difficult to explain. In race 56 there is some evidence that its increase in prevalence was, at least in part, due to the susceptibility to it of the wheat variety Ceres which was widely grown for some years in the spring wheat areas of the United States (100, 190). The increased susceptibility that this variety showed as race 56 became more and more predominant is a good example of the practical importance that particular physiologic races may assume. It is, of course, obvious that the increase in the abundance of any physiologic race is conditional on the presence of susceptible hosts, but there are other factors that are also of importance. One of these is the length of the period of urediospore production. Physiologic races differ greatly in this respect. Some produce urediospores for only a short period before the development of teliospores sets in. Others, and these include the most prevalent races, remain for much longer periods in the uredial stage before they begin the formation of telia—a characteristic that obviously favors their spread in any season (94, 144). Races differ also in their ability to develop under adverse conditions of temperature (23, 96, 134), and they may also vary in ability to survive winter weather, a subject on which little is known at present.

Physiologic specialization in Puccinia graminis Avenae

In contrast to the large number of physiologic races in the variety *Tritici,* only 13, or possibly 14, races are known in the variety *Avenae*. Stakman, Levine and Bailey (194) in 1923 described four distinct physiologic races of oat stem rust. They inferred the existence of a fifth race which was later described by Bailey (8). These five races were distinguished by the infection types produced on three differential oat varieties, White Tartar, Richland and Joanette Strain. Only three of these races (races 1, 2 and 5) were collected in North America, where they, however, were widely

distributed. Race 3 was found in rust collections from Sweden and South Africa, and race 4 occurred only in a collection from Sweden. Gordon and Bailey (73) collected all these races in Canada in 1925 as well as a sixth race (race 6). Two new races, race 7 (originally numbered 6) and race 8 (originally numbered 7), were reported by Waterhouse (234) from Australia in 1929. These two races were also found in Canada (74) about the same time, as was also a ninth race, race 9, which was first isolated there in 1930. Race 10 was discovered in the United States (26) in the same year, and race 11 was first collected in Germany in 1937 (83). Races 12 and 13 were first found in Canada (150). The latter originally occurred in cultures derived from artificially inoculated barberry and was collected in the field for the first time in 1944. A possible fourteenth race may be represented by a culture of stem rust collected in America on *Poa ampla* (49). As, however, it attacks several species of *Poa* and as all but one of the 17 varieties of cultivated oats tested proved immune, there is perhaps equal reason to regard it as a physiologic race of *Puccinia graminis Poae.*

When the distribution of the races is considered, it becomes evident that there are striking pathogenic differences in oat stem rust in different parts of the world. In North America races 1, 2 and 5 have predominated since the first studies on the specialization of this rust were made—a fact highly advantageous to plant breeders, as these races do not attack the varieties Richland and White Russian which have been used extensively as parents in breeding for resistance against oat stem rust. In Europe, as far as can be judged from the rather limited surveys conducted, other races with a wider range of pathogenicity are more common. Tedin (216) has reported the presence in Sweden of races 3, 4, 6, 7 and 8. Hassebrauk (81) studied six collections of rust from Germany and one from Bulgaria and found them to consist entirely of race 6. Later (83) he studied 13 collections from southern Germany and identified races 1, 2, 4, 6, 8 and 11. From his studies it would appear that the most common race is the highly virulent race 6 which attacks all oat varieties except a few produced recently that have not yet been distributed for cultivation. In South Africa only races 3 and 7 appear to have been reported (228), and in the Argentine the same two races are known to be present (227). In India races 3, 4, 6 and 7 have been identified (133), and in Australia the

presence of races 1, 2, 3, 6, 7 and 8 has been established (239). Races 1 and 2 were by far the most common in Australia where they occurred annually from 1925 to 1938. They were also found in collections made in New Zealand. Races 3 and 7 were not uncommon in these two countries in certain years, but races 6 and 8 were rarely collected.

The question of whether the same races persist over a period of years in the same region is one of considerable practical importance, but it is a question that can be discussed only for regions in which yearly surveys have been conducted for a considerable time. Only in North America (the United States and Canada) and in Australia have annual surveys been carried out for periods long enough to warrant discussion. In North America races 1, 2 and 5 have maintained their predominance since surveys for races of oat stem rust were first undertaken, about 1921, and there is as yet no definite indication of their displacement by other races, although in 1942 and 1943 there was a notable increase in the prevalence of races 8, 10 and 11 (150, 200). In Australia (239) races 1 and 2 have been the predominant races throughout the period 1925–1938, with races 3 and 7 holding a rather distant secondary place. In 1938, the last year for which data are at hand, a considerable increase took place in the proportion of the last mentioned races—an increase that would, if maintained in succeeding years, make these races of much greater importance than previously. Despite these indications there is no conclusive evidence of great fluctuations in the prevalence of the various races of oat stem rust comparable at all with those already recorded for races of *P. graminis Tritici*.

Physiologic specialization in Puccinia graminis Secalis

One of the most essential qualities of good differential varieties is homozygosity of the plant material of each variety, which ensures uniformity of the rust reaction of the plants. In cereals that are self-pollinated it is easy by means of periodical selection to maintain the desired purity of each differential host. In a cross-pollinated crop, such as rye, the investigator of the specialization of its rusts is faced with the difficulty of securing lines with even a reasonable uniformity of reaction to rust, and, having once secured such a line, he encounters further difficulty in maintaining its purity.

Levine and Stakman (119), however, found that certain commercial varieties of rye reacted clearly enough to indicate that there were two and probably three distinct physiologic races of *Puccinia graminis Secalis*. One race possessed the ability to attack heavily the varieties Rosen, Swedish and Prolific. A second race attacked Rosen heavily, Prolific moderately and Swedish weakly; and a third race produced moderately heavy infection on Swedish and Prolific but weak infection on Rosen.

Later, Cotter and Levine (28) identified 14 physiologic races from 147 collections made in the United States, Canada, France, Scotland and Sweden. They used as differential hosts the varieties Rosen, Swedish, Prolific, Dakold and Colorless. The heterozygosity of the plant material necessitated the testing of large numbers of plants of each variety—an average of 200 plants being infected with each culture of the rust. A variety was considered resistant if more than 75% of the plants developed infection types 0, 1 or 2; mesothetic if less than 75% of the plants developed infection types 3 or 4, or when infection on all or most of the plants was type x; and susceptible when 75% or more of the plants showed types 3 or 4.

Of the 14 races identified, all were found in the United States except race 6 which was collected in Sweden. The bulk of the isolates from the United States, almost 60%, was made up of races 7 and 11. Collections from the other countries were too few in number to provide information on the predominance of any particular race.

Outside of the work reviewed above, the specialization of this rust has received but little attention. Verwoerd (228), however, states that two physiologic races of *P. graminis Secalis* are present in South Africa.

Physiologic specialization in Puccinia triticina

Mains and Jackson (125) in 1926 demonstrated the existence in *P. triticina* of 12 physiologic races which they distinguished by the reactions of 11 wheat varieties of which eight have since come to be generally accepted as differential hosts for this rust. Since then work on the determination of physiologic races has been undertaken in many countries, and new races have been described (7, 9, 21, 38, 105, 132, 171, 174, 175, 184, 186, 187, 230, 231, 236) in practically all regions where wheat is an economically important crop.

As there have been a greater number of separate and independent studies on the specialization of this than of any other cereal rust, it is not unnatural that a considerable confusion should have arisen with respect to the numbering of new races. Frequently investigators have assigned certain numbers to new races in ignorance of the fact that the same numbers had been used elsewhere for different races or that the same race had been described elsewhere under a different number. This confusion has, as far as possible, been clarified by periodical revision of the numbering of races described by individual investigators[3]. The last such revision contains 129 distinct races, but this is probably short of the actual number of races described up to the present time.

In this rust the number of physiologic races known to be present in different regions varies very considerably. In North America about 70 races have been collected up to the present, and in Europe a large number of races are known to exist. In India only six races were collected in the eight-year period 1930–37 (133), and in Australia only two races were identified from more than 1200 collections made during the period 1927–1938 (239).

The reasons for these striking differences in degree of specialization are not clear. Possibly the diversity of physiologic races in Europe is due to the presence there of susceptible species of the aecial host, *Thalictrum* spp., which occurs commonly in southeastern Europe and is often heavily rusted (171). Despite the occurrence of species of *Thalictrum* that have been shown to be susceptible in artificial infection studies, investigators have generally failed to prove that the aecia occurring in nature on these species are connected with *P. triticina* (39, 40, 140, 173, 183, 184, 218). D'Oliveira (156), however, has reported the presence under natural conditions of aecia of this rust in Portugal on *T. flavum* var. *glaucum*. With reference to North America, Arthur (5) states that no field collections of aecia have been identified with *P. triticina* but ventures the opinion that such undoubtedly do occur. In India there is, according to Mehta (133), no evidence that native species of *Thalictrum* bear aecia of this rust. No information

[3] These revisions have been done by leading authorities on leaf rust in the United States. The most recent is the "Third Revision of the International Register of Physiologic Races of Leaf Rust of Wheat (*Puccinia rubigo-vera tritici*)", by C. O. Johnston, H. B. Humphrey, R. M. Caldwell and L. E. Compton. April, 1942. (Mimeographed for private distribution.)

is available to the writers on the presence of species of *Thalictrum* in Australia, but, as only two races of leaf rust are known to occur in that country, it would appear that an alternate host, if present, does not function actively. That it could so function was shown by Waterhouse (236) who infected two species of *Thalictrum* with the two Australian races and from the aecia so produced isolated these two races and two others previously unknown.

It is difficult to decide whether or not there are any pronounced pathogenic differences between the leaf rust of wheat present in different regions of the world, partly owing to the absence in many of these regions of surveys conducted over periods of years and partly owing to frequent lack of agreement between the findings of different investigators working in the same geographic area. There are, however, indications that certain races, or races with certain pathogenic characteristics, tend to predominate in some geographic areas. Races 1 and 11, which do not attack the variety Malakof, occur most frequently along the Pacific coast of the United States and Canada (105, 125, 149), and the former of these races has been found frequently along the Atlantic seaboard. Neither one occurs commonly in the interior of the continent. In the Mississippi Valley races 9 and 13, which attack Malakof heavily, are common but not much more so than certain other races that do not attack this variety (24, 105). In Europe a great variety of races, some of which attack Malakof and some of which do not, have been identified in the interior regions, whereas in Great Britain, Holland and Italy the great majority of the races present are incapable of attacking this variety (175, 186, 187, 220, 241).

There is no evidence of any great changes from time to time in the prevalence of the various races of this rust that are in any way comparable to the fluctuations already reported for races of wheat stem-rust. Nevertheless new physiologic races appear now and then and gain wide distribution, as exemplified by the present prevalence in the United States and Canada of race 128 which was unknown until a few years ago.

Physiologic specialization in Puccinia dispersa (*P. rubigo-vera Secalis*)

Probably the first indication of the specialization of this rust is contained in a report by Mains (121) of the existence of two

pathogenically distinct strains which he distinguished by the reaction of an inbred line of rye. This line was susceptible to one culture of the rust and resistant to another, thus indicating the presence of two physiologic races. An investigation into the specialization of this rust was also carried out by Gassner and Kirchhoff (60). On the rye varieties tested, the cultures that were studied showed some slight pathogenic differences that were, however, scarcely significant enough to permit the differentiation of physiologic races. Tests with wheat varieties showed that, despite their very high resistance to this rust, it was possible to detect symptomatic differences between cultures. Of the 10 cultures studied, one was distinguished by its inability to cause necrotic lesions on the leaves and was, therefore, considered sufficiently distinct pathogenically to be regarded as a different physiologic race. The same culture also differed from the others by its tendency towards early formation of teliospores on rye.

Further evidence on the specialization of this rust is submitted by Waterhouse (240). He observed susceptible and resistant reactions on one and the same leaf of a rye plant and was able, through the selfing of selected plants, to produce plants that indicated by their rust reactions the presence in Australia of at least two physiologic races.

Physiologic specialization in Puccinia glumarum

From 1925 to 1928 Rudorf (178) tested the reaction of a number of wheat varieties to *P. glumarum* in Germany and found that some of the varieties that had been classified by Hungerford and Owens (91) as resistant to this rust in America were susceptible in Germany. He concluded, therefore, that the stripe rust occurring on wheat in Europe differed pathogenically from that in America. This conclusion soon found support in the work of several investigators. Allison and Isenbeck (2) in 1930 demonstrated the presence of four physiologic races in European stripe rust collections on wheat; at about the same time in Germany, Gassner and Straib (63) reported the occurrence of two physiologic races, and Appel (3) was able to distinguish five races. Wilhelm (242) a few months later reported the isolation of five physiologic races from rust collected in Germany, France and Sweden.

As these investigators worked independently of each other, their

respective physiologic races were identified by means of different groups of differential hosts. Subsequently Gassner and Straib (65, 67, 69) developed a group composed of 11 wheat varieties to which were added later (206, 211) supplementary varieties of wheat, barley and rye. By means of these differential hosts 47 physiologic races of stripe rust were identified by 1939 in Europe (211, 214), exclusive of several races described by Becker (12, 195). Of these 47 races, 37 were regarded as specific to wheat, four to barley, two to *Agropyron repens,* one to rye, one to *Hordeum murinum* and one to *Hordeum jubatum.* The specificity of these races, however, was not great enough to permit them to be classified as varieties of the rust in the sense used by Eriksson (41).

Though *P. glumarum* has been found in many parts of the world, its development is favored by a cool or moderate climate in which the summers are not excessively hot. The economic importance of stripe rust is probably greater in northern Europe than that of any other rust. It is for this reason that most of the work on the specialization of this rust has been done in Europe and particularly in Germany where it is widespread and most of the varieties commonly grown are susceptible to it (210).

In North America the rust is common and occasionally destructive along the Pacific coast and in some of the inter-mountain regions of the United States and in certain areas of Mexico (89, 90), but is largely restricted in its eastward spread to the areas immediately east of the Rocky Mountains (90, 145, 180, 181). Only a limited amount of work has been done on the specialization of stripe rust in America. Bever (14) in 1934 established the presence in Montana and Idaho of two physiologic races which he did not at that time classify as to number but later designated as races 19 and 28[4]. Newton and Johnson (145) found races 8 and 13 in rust collections from western Canada, the latter race, which resembled Bever's race 28, being the common race in that region. Previously, however, race 8 had been collected many times in Europe, but race 13 had not been found elsewhere.

In South America stripe rust is prevalent in Chile, the Argentine and Uruguay (89, 126, 179), and Straib (212) has recorded the presence there of four physiologic races (races 30, 37, 38 and 39) that differ pathogenically from any of the races found in Europe.

[4] Private communication to the writers. The race 28 identified by Bever is described under that number by Stakman *et al.* (195) but is not identical with race 28 described by Straib (211).

On the continent of Asia work on the specialization of *Puccinia glumarum* has been reported by Mehta (133) in India and by Fang (48) in China. During the period 1932 to 1937 Mehta identified, from Indian collections on wheat and barley, races 13, 19, 20 and 31, as well as four new races designated as A, D, E and F. His results indicate that the physiologic races in India differ from those in Europe and America, although race 13 had been found previously in Canada and races 19 and 20 had been collected in Turkey and Bulgaria. Race 31 had previously been found only in adjacent Afghanistan. The predominant race in India was the new race A, races 31 and 19 being next in importance. The other races occurred only rarely. In China, Fang identified nine physiologic races from 43 collections made chiefly in the province of Yunnan. These races cannot be compared directly with any collected elsewhere, owing to the fact that he did not use several of the differential hosts now in common use as differential varieties. A striking feature of the Chinese races, which distinguishes them from most races collected in other countries, is their ability to attack the wheat variety Chinese 166 which is susceptible to only three of the 47 races described by Straib (211, 214).

It seems clear from the work done thus far that not only does this rust possess a high degree of specialization but that this specialization differs in the different regions in which the rust is prevalent. Thus, for example, race 7, which is the most common race in Germany (210), has not been found outside Europe; race 13, the common race in Canada, has not been reported elsewhere, except rarely in India; the races reported from South America appear to be restricted to that region; and the most common race in India has not been discovered anywhere else.

Physiologic specialization in Puccinia coronata Avenae

Hoerner (88) in 1919 established the presence of physiologic races within the *Avenae* variety of *P. coronata* through the infection types developed on two oat varieties (Ruakura and Green Russian) by means of which he was able to distinguish four physiologic races. Within the next ten years investigators in the United States, Canada and Germany proved that this rust comprised numerous pathogenically distinct strains. Popp (170) studied a number of rust collections made in Canada and distinguished four races

through the reaction of three oat varieties. Parson (159) identified five races by the reaction of four varieties, and Murphy (137), using eight differential varieties, identified nine races. Peturson (167) reported the presence of eight physiologic races in Canada.

From the varieties which these investigators used for determining physiologic races, Murphy (138) selected 11 that have since come into general use in America and have been adopted in several other countries. By means of these he was able to identify 33 races from collections made in the United States, Canada and Mexico from 1927 to 1932. Using the same varieties Peturson (168) determined the presence in Canada of nine of these races and two additional ones not previously described. Through the use of the varieties selected by Murphy, at least 82 physiologic races have been identified up to the present time[5].

A comparison of the races identified in different parts of the world shows that specialization of the rust is somewhat dissimilar in widely separated regions. In North America 75 races have been determined, namely, races 1–39, 41, 45, 46, 48–54 and 57–82, the predominant races being 1, 2, 3, 4 and 6. In South America, Vallega (224) has demonstrated the presence of races 1 and 45 as well as two new races 55 and 56 which have not been found elsewhere. He found that race 1 was widely distributed and common in the Argentine (as it also is in North America) but that the other three races were rarely found despite their wider pathogenic range. In Australia, Waterhouse (240) has reported the presence of races 3, 6, 40 and 47. The first three are known in America but the last two have not been found outside of Australia. In England, Brown (18) has identified races 6, 42 and 44, and race 43 collected in Portugal. Race 6 occurs commonly in North America and, as shown above, was found also in Australia, but the other races had not been discovered elsewhere.

It is not possible to make any direct comparison of the races identified in continental Europe with those reported elsewhere, owing to the employment there of other groups of differential hosts. Frenzel (54) studied collections of crown rust made in Germany and adjacent countries and, by the use of nine differential hosts, including four that had been used by workers in America, was able to distinguish 33 physiologic races. Straib (209), in a study of

[5] Information supplied by Mr. B. Peturson.

144 uredial collections made in Germany, identified 139 physiologic races and three additional ones from aecia on *Rhamnus cathartica*. These races he determined by the use of 15 differential varieties that included several not used by investigators in America. The greater number of races identified in Germany than in America does not necessarily prove that crown rust there is much more highly specialized, as it is well known that an increase in the number of differential hosts used is likely to increase the number of races determined. In fact, Kingsolver and Murphy (106) studied a group of 81 single-pustule isolates which, when identified by means of the American differential hosts, were resolved into 19 races but comprised no less than 46 races when identified by means of Straib's differential hosts. Moreover, some of the German races were identified by the use of somewhat finer gradations than are ordinarily employed by American workers.

In the absence of a direct comparison of European and American races, it is still possible to compare some of their characteristics because the German workers used certain of the American differential hosts. In this connection the reactions of the varieties Bond and Victoria are significant. In North America, Bond is susceptible, as far as is known, to only six (or 8.0%) of the 75 races thus far collected, whereas it is susceptible to 36 (or 25.4%) of Straib's 142 races. Similarly Victoria is moderately susceptible to four (or 5.3%) of the 75 American races but is moderately susceptible to 45 (or 31.7%) of the German races. Although Straib's results are based chiefly on collections made in Germany and represent only a two-year survey, the comparisons indicate nonetheless a pathogenic difference between European and North American strains of crown rust.

Physiologic specialization in Puccinia anomala

The existence of physiologic races in *P. anomala* was first shown by Mains (121) who found three varieties of barley, Oderbrucker, Featherstone and Horsford, highly resistant to one culture of the rust and susceptible to another, and thereby established the existence of two physiologic races. He later (122) developed a group of nine differential varieties by means of which he repeatedly identified these two races from rust collections made in the United States. Although only these two races were found in America, he obtained

evidence of a third race in Australia through tests that demonstrated that certain barley varieties shown by Professor Waterhouse to be resistant in Australia were susceptible to the American races.

Brown (16, 17), in a study of rust collections of Canadian origin, identified four physiologic races by means of the reaction of six barley varieties; but, as all his varieties were different from those used by Mains, it is not possible to make any direct comparison among the two groups of races.

Hey (86), by means of yet another group of varieties, identified eight physiologic races from 17 rust collections made in Germany. Ronsdorf (176), using Hey's differential hosts and one additional barley variety, identified a ninth race, also from Germany, and later (177) described two additional races derived from an American collection, thus bringing the total number of races identified by means of Hey's differential hosts to eleven. She also tested five of the German races for their infection types on Mains' differential hosts and found them to behave like one and the same race, from which she concluded that the set of differential varieties developed by Hey was better adapted for the differentiation of physiologic races than those selected by Mains.

D'Oliveira (155), using seven of the ten hosts selected by Hey, the one added by Ronsdorf and two of those employed by Mains, described 11 new physiologic races from collections made in Great Britain, Portugal and Spain. In addition he described seven new races that arose as a result of the selfing (on *Ornithogalum umbellatum*) of two known races, one new race that arose from crosses between two known races, and one new race that originated in the mutation of a race previously collected in England. At least 30 physiologic races of *P. anomala* are, therefore, known, exclusive of those identified by Brown and Mains, through the use of other assortments of differential hosts. The hosts developed by Mains have been utilized by Waterhouse (238) in Australia for a study of the specilization of the rust in that country. The results indicated the presence of a race similar to though not identical with Mains' race 1.

Owing to the fact that different groups of differential hosts were used by North American and European workers, it is not possible to make any comparison between the races present in the two continents. Ronsdorf (177), however, ventured the opinion that the American races probably differed from those in Europe.

According to the work thus far done in Europe, it is probable that specialization in *P. anomala* is quite extensive. In this respect it is probably significant that d'Oliveira (155) did not isolate from collections made in England, Spain and Portugal any of the races collected by Hey or Ronsdorf in Germany. Moreover, he found only one race (race 12) that was distributed widely in both Great Britain and the Iberian peninsula; other races collected were 13, 14 and 15 in Great Britain, 18, 19, 20 and 21 in Portugal, and 16, 17, 21 and 22 in Spain.

Reaction of physiologic races to environment

It was early realized that each of the cereal rusts was best adapted to a particular environment. Stripe rust (*P. glumarum*) develops best in comparatively cool weather and flourishes in early summer in northern Europe and in other regions with a moderately cool growing season. Leaf rust of wheat (*P. triticina*) is adapted to somewhat higher temperatures, and stem rust (*P. graminis*) is favored by the comparatively high temperatures of mid-summer (162). Adaptations to conditions of moisture or of light are less obvious; in regard to stripe rust, which commonly develops in the fall and spring seasons, it is, however, possible that the abundant moisture and dull weather of those seasons are factors particularly favorable to this rust.

In view of these environmental adaptations, it is not surprising that the physiologic races of the various rusts should respond rather readily to the environment to which they are exposed in greenhouse studies. Each rust has its optimum conditions of temperature and perhaps also of light, and any marked deviation from this optimum is reflected in its development. In general, a rise in temperature shortens the incubation period—the time elapsing from infection to the outbreak of uredia (52, 61, 139, 160); but it may also have other effects. For example, in stripe rust, which requires cool weather for satisfactory growth, a high greenhouse temperature will render the host resistant and may even completely inhibit rust development (62, 145). Obviously this influence of temperature is of great importance in the determination of the physiologic races of this rust, as the infection types, by means of which races are determined, are strongly affected. The same conditions that influence the expression of this rust in the greenhouse will determine

its behaviour in the field. It has been shown, for instance, that wheat varieties that are susceptible to it during the cool weather of early summer tend to develop a considerable amount of resistance on the advent of warm summer weather (13, 68, 109, 172, 215). Temperature conditions also have a pronounced effect on the distribution of stripe rust. It has been suggested (145) that in western Canada, where there is a limited spread of stripe rust each spring in an easterly direction from the foothills of the Rocky Mountains, the high day temperatures in the summer months check the growth of the rust and thereby tend to restrict its eastward distribution. In tropical or sub-tropical regions this rust thrives chiefly in the cooler conditions of high altitudes. In Kenya (British East Africa) it is prevented from spreading to low altitudes by the higher temperatures that prevail there (127). In India it survives the summer in mountainous districts and spreads down into the plains during the cooler weather of winter (130, 131, 132, 133).

The other cereal rusts, though somewhat less reactive to temperature, are sufficiently influenced to make it imperative that close attention be paid to that factor in the identification of physiologic races. It has been shown for stem rust of wheat (94), stem rust of oats (71, 72) and crown rust of oats (166) that physiologic races which under optimum conditions of temperature produce the X type of infection, may, at a higher temperature, produce the 4 type. In terms of host reaction this means that a mesothetic reaction may be changed to a susceptible one. The consequence might be that the same culture of rust would be identified as one physiologic race at a moderate temperature and as another race at a higher temperature. In the routine work of identifying physiologic races this sensitivity to environment is likely to result in different reactions on the part of certain host varieties at different seasons of the year (125, 234), unless important factors of the environment, such as temperature and light, are under effective control.

In leaf rust of wheat (*P. triticina*) and leaf rust of barley (*P. anomala*) the influence of temperature is complicated by the fact that the reaction of different host varieties is affected quite differently by this factor. It has been shown that the reaction of certain of the wheat varieties used as differential hosts for *P. triticina* tends to become increasingly susceptible with lower temperatures (64,

84), while that of some other varieties becomes increasingly susceptible with higher temperatures (84, 145). In some varieties the degree of change in reaction was found to depend on the physiologic race used (84). Much the same sort of relationship appears to exist between temperature and the reaction of certain of the barley differential varieties to *P. anomala* (86, 177).

Some of the other variable factors of the environment exert an influence on rust development second only to that of temperature. Deviation from optimum conditions of light should be avoided, as far as possible, in the determination of physiologic races, as deviations therefrom may alter considerably the rust reaction of a host. Subnormal conditions of light, in particular, generally tend to shift the reaction to rust in the direction of greater resistance (84, 94, 149, 161, 175) and to lengthen the period of incubation (50, 53, 85, 120, 134, 204). Excess of nitrogenous fertilizer influences rust reaction in the direction of increased susceptibility, whereas a deficiency of it or an excess of potash has the opposite effect (36, 57, 58, 59, 78, 158). Superabundance of soil moisture is reported to increase chlorosis and diminish the size of rust pustules (80). All the above-mentioned factors exert their influence most strongly on the reaction of plants that show a moderate degree of rust resistance, or a mesothetic reaction, while the reactions of plants that are immune, highly resistant or susceptible are usually affected less and often not at all.

Economic significance of physiologic races

The economic importance of physiologic specialization lies in the fact that the existence of many physiologic races in a rust confers on it a pathogenic versatility whereby the rust may attack a wide range of host varieties even if each individual race possesses only a narrow host range. It is not always possible for the plant breeder concerned with the production of resistant cereals to develop agronomically satisfactory varieties that are resistant to all the physiologic races of a rust. Where this is not possible, emphasis is usually placed on producing varieties resistant to the predominant races of the region in which the varieties are to be grown. If in this region there still exist physiologic races capable of attacking these varieties, the danger remains that these races, though perhaps previously unimportant, may assume greater prevalence and impor-

tance; and the more intensively these varieties are grown the greater is the danger that they may exert on the rust a selective influence favoring the increase of those races to which they are susceptible. It has indeed been maintained (76, 190) that such a selective action on the part of the wheat variety Ceres led to the rise to prominence in North America of race 56 of *P. graminis Tritici.*

Even when the plant breeder has been successful in producing varieties that seem to be resistant to all the physiologic races present, as appears to be the case in the production in the United States and Canada of varieties such as Thatcher, Pilot, Renown, *etc.,* there is still no guarantee that new physiologic races capable of attacking them may not arise. The appearance in the last few years in the United States of race 15 B—a biotype of race 15 capable of attacking these new varieties—is a potential threat to many of the recently produced rust-resistant wheats and an example of the emergence of a previously unknown strain of wheat stem rust (75, 76, 201).

The plant pathologist and the plant breeder responsible for developing rust-resistant varieties must face the fact that new physiologic races with pathogenic traits different from those of known races may arise at any time, particularly in regions where the presence of the alternate host enables crossing and selfing of existing races to take place. It must be admitted that, by virtue of the production of ever new physiologic races, the rust possesses a very considerable power of adjustment to its host varieties—a power that could obviously be greatly diminished by the eradication of the alternate hosts. Even, however, if the alternate hosts were destroyed, it is not safe to assume that new physiologic races could not arise. As far as can be seen at present, the price of security from rust damage is a continuous vigilance in the form of surveys to detect any pathogenic changes in the economically important rusts and unrelenting efforts to develop cereal varieties resistant to any virulent races that may arise.

HYBRIDIZATION IN CEREAL RUSTS

Nuclear phenomena

Before hybridization can be discussed intelligibly it is necessary that the nuclear phenomena involved in the different phases of the

life cycle of the rust be understood. According to Arthur (4), the cytology of the rusts may be regarded to have commenced about 1880 with the observation by Schmitz (185) that the nuclei in the urediospores and mycelium of *Coleosporium Campanulae* (Pers.) Lev. were arranged in pairs. Later, Poirault and Raciborski (169) gave the paired nuclei the name "conjugate nuclei". Sappin-Trouffy (182) studied the behavior of the nuclei in many rust species. He established that the pycniospores and the hyphae from which they develop are uninucleate, but that the aeciospores, urediospores and the mycelia arising from them are binucleate. The binucleate condition persists in the immature teliospores; but in the mature teliospore a fusion of the two nuclei takes place. On the germination of the teliospore, four nuclei are produced as a result of two successive nuclear divisions of which one is a reduction division. These nuclei migrate into the developing sporidia (basidiospores) so that, when newly formed, the sporidia are uninucleate. Infection of the alternate host by the sporidia is soon followed by the development of the pycnia beneath which are formed aggregations of uninucleate mycelium that have been given the names "aecial primordia" (1) and "protoaecidia" (22), here referred to as "protoaecia". From these mycelial aggregations are later developed the binucleate aeciospores. The transition from the uninucleate to the binucleate condition was regarded by Blackman (15), Christman (25) and others as involving a sexual fertilization. The mechanism of this process, however, remained unknown until Craigie's discoveries that certain rusts were heterothallic (29, 31) and that the pycnia, which had previously been regarded as functionless, performed a definite function (30). He showed (32) that when pycniospores of *Puccinia graminis* and *P. Helianthi* Schw. are transferred from one pycnial pustule to a number of other pustules, aecia are formed beneath about one half of the pustules receiving the transfer of pycniospores; whereas, if no transfer is made no aecia are formed. Two important deductions may be made from his experiments: First, that the pycniospores are the bearers of one of each pair of the nuclei that become associated in the young aecium; second, that the pycnia may be classified into two different "sexual"[6] or intersterility groups. These groups he designated by the symbols (+) and (−).

[6] Objection has been raised to the term "sexual" as applied to these groups on the ground that the pycnia and protoaecia are to be regarded as male and

Discovery of the function of the pycnia provided at once a means of bringing about the association of the nuclei of one physiologic race with those of another. In other words, it made possible the "crossing" of physiologic races.

Methods of crossing and selfing

Methods of crossing and selfing physiologic races of *Puccinia graminis* have been described (144, 152). In crossing two physiologic races, the pycnial exudate from a pustule (presumed to be of monosporidial origin) of race A is applied successively to several other similar pustules of race B. The resulting aeciospores contain one nucleus of race A contributed by a pycniospore and one of race B contributed by the uninucleate mycelium at the base of the pycnial pustule. The process by which the pycniospore nucleus reaches its destination in the young aecium has been discussed by Craigie (33) and Buller (22). The selfing of a physiologic race involves merely the intermixing of the pycnial exudate of pustules of the same race to bring about the various possible nuclear combinations.

The great bulk of the work on hybridization in the rusts has been carried out with the economically important species *Puccinia graminis* Pers. which, though regarded as a morphological unit, is composed of several physiologically distinct subspecies originally designated by Eriksson (41) as *formae speciales* but here referred to as varieties. Each of the three varieties that are pathogenic to cereals (*P. graminis Tritici, P. graminis Avenae, P. graminis Secalis*) comprise, in turn, a number of physiologic races. In crossing strains of *P. graminis,* two kinds of crosses are therefore possible, crosses between the different physiologic races of a single variety (intra-varietal crosses) and crosses between physiologic races of one variety and those of another variety (inter-varietal crosses). In the present paper these two phases of hybridization will be discussed separately.

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female organs, respectively. As these organs are present in the haploid thalli of both (+) and (−) groups, it is argued that each thallus is hermaphroditic but self-sterile. As, however, the pycniospores of one group can fertilize the protoaecia of the other, the two groups are cross-fertile but the cross fertility is dissociated from sex, the factors for (+) and (−) therefore being interfertility factors. Functionally, the (+) and (−) elements act as gametes, for in a "cross" between two physiologic races they bring together the haploid nuclei of the two "parents" to form a dikaryotic and finally a truly diploid condition.

Investigators have, in general, kept in mind two main objectives, first, to compare the pathogenic characteristics of the hybrid (F_1) race or races with those of the parental rust races used in the cross, and second, to study the inheritance, in the first and in subsequent generations, of any observable characteristics of the rust. To accomplish these aims it is necessary to have an exact knowledge of pathogenic and other characteristics of the parental strains; and the parental strains should, furthermore, be kept available for comparison with the hybrid rusts. When a study is to be made of the inheritance of rust characteristics in later generations, the hybrid rusts must be grown in pure culture in the uredial stage and then allowed to complete the life cycle by the production of teliospores, which must be induced to germinate and infect barberry (*Berberis vulgaris* L.), the alternate host of stem rust. In the reduction division that occurs during the germination of the teliospore, segregation of genes takes place so that these become variously distributed among the haploid sporidia and consequently also among the haploid infections produced by these sporidia on leaves of the barberry. After the haploid infections have produced pycnial exudate, the process of selfing is carried out by intermixing the pycniospore-bearing exudate of the different haploid pustules. By this process new associations of nuclei (and, therefore, of genes) are brought about in the binucleate aeciospores of the new generation of rust. Study of the strains of rust appearing in the new generation is primarily a study of the effect of these nuclear associations. As mass cultures established from numerous aecia would produce a mixture comprising several strains of rust, investigators usually initiate cultures from individual pustules or preferably from individual aecial cups. It has been established (152) that approximately 95% of cultures started from individual aecia contain, each, a single physiologic race.

Rust characteristics suitable for study

Before a discussion of experimental results is begun it is advisable to mention some of the chief rust characteristics that have proved amenable to study. It is obvious that no genetical studies can be conducted with an organism unless it possesses some properties that may be regarded as characters. In *Puccinia graminis,* morphologic characters are not available for study because the

spores (aeciospores, urediospores, teliospores) of the various physiologic races within a variety, and indeed also the spores of the different varieties, appear to be virtually identical. The physiologic races are based entirely on physiological, *i.e.,* pathogenic, differences. Nevertheless, the pathogenic criteria—the infection types—are visible phenomena and are characteristic for each physiologic race. Thus, race 14 of *P. graminis Tritici* produces on seedling leaves of the variety Marquis a minute rust sorus about the size of a pinhead, surrounded by a halo of necrotic tissue (type 1); whereas race 15 produces on the same host a large elongated sorus that may attain a length of half a centimeter or more and is not surrounded by necrosis (type 4). By selfing these races it is possible to discover whether or not they are homozygous for the respective characters. If they prove homozygous—that is, if all the progeny of the selfing study reproduces the character in question without appreciable variation—it is possible to cross the two races with good prospects of learning whether one of these infection types is dominant over the other or whether the hybrid rust is characterized by some entirely different type of infection. In such a cross all the pathogenic differences shown by the two physiologic races may be studied one by one. For example, the two races mentioned above differ also by the infection types produced on the wheat varieties Kanred, Kota and Vernal, on which race 14 produces minute necrotic spots or small pustules of the 1 type whereas race 15 forms large sori of types 3 and 4.

Spore color, particularly the color of the urediospores, is another character that has proved amenable to study. Though some differences of color do exist in the uredial stage of stem rust collected in nature (143, 234), these are usually too slight to afford a satisfactory basis for study. Strains of strikingly abnormal color do, however, occasionally arise, presumably by mutation. Newton and Johnson (143) reported two such deviations from the normal red color of the uredia. One of these consisted in the production, among the red uredia of *P. gr. Tritici* race 9, of a few sori of orange color from which originated an orange strain of this race. The other consisted in the development from aecia of a strain of race 36 with greyish-brown uredia. Stakman, Levine and Cotter (196) likewise developed from aecia three strains of *P. gr. Tritici* race 58 described as Argus Brown, Dull Auburn and Mars Yellow[7]. Waterhouse

[7] Ridgway's Color Standards and Color Nomenclature.

(234) and Gordon (72) obtained strains of *P. graminis Avenae* with orange uredia from aecia on barberries inoculated with telia gathered in the field. As the uredial color of these strains is readily distinguishable from that of normal rust, the color changes provided investigators with visible characters whose inheritance could be studied in selfing and crossing experiments.

Deviation from the normal in color of the aecia has also been noted. Aecia of strains with red or orange urediospores were orange in color, while aecia of strains with greyish-brown urediospores were a pale yellow (153.) In *P. graminis Agrostidis,* white pycnia and aecia have been found (27).

The fact that different physiologic races of the same rust vary greatly in their readiness to produce teliospores—some producing them rapidly after a short interval in the uredial stage, others only slowly after a long interval—is another rust characteristic that may be subjected to inheritance studies.

Although the above characteristics all pertain to the binucleate or dikaryotic phase of the rust, it does not follow that there are no characters of the haploid phase that can be made subject to inheritance studies. Morphologically, however, the pycnia of different races appear to be much alike, and consequently the only haploid characters that thus far have been studied are certain abnormalities that have arisen, such as the production in some physiologic races of white (chlorotic or necrotic) haploid infections with few pycnia and little or no capacity to develop aecia.

Selfing of physiologic races

Among the first attempts at genetic studies in the rusts was the selfing by Newton, Johnson and Brown (152) of several physiologic races of *P. graminis Tritici.* Of eight physiologic races studied, only one proved to be homozygous for all of the various pathogenic characteristics expressed on the 12 differential wheat varieties used for determining physiologic races. This and later studies (98) have shown that the majority of physiologic races of this rust are heterozygous for pathogenic characters. Frequently, however, a race heterozygous for certain pathogenic characters is homozygous for others—which shows that more than one pair of genetic factors are operative. Thus a race may be homozygous for the infection types developed on all but two or three differential hosts, and yet

the heterozygosity for the infection types on these two or three varieties may lead to the production of several physiologic races in the progeny of a selfing.

Selfing studies soon produced information indicating the dominance or recessiveness of certain infection types. For example, races which do not rust the variety Kanred (0 type of infection) fall into two groups (98). Some produce, when selfed, only races that do not rust Kanred; that is, they are homozygous for that character. Others produce, in addition to such races, other races that rust it heavily (4 type of infection)—a fact indicating that inability to rust this variety (0 type) is dominant to the ability to rust it heavily (4 type). It follows that races phenotypically alike are not necessarily alike genotypically; or, in other words, two different cultures of the same race do not necessarily, when selfed, produce identical progeny. An example is afforded by the occurrence in the progeny of a cross between races 9 and 52 of two genetically distinct types of race 15, one of which was homozygous, producing only race 15 when selfed, whereas the other was heterozygous and produced a progeny consisting of races 15 and 52 (103).

Little work has been done in rusts other than *Puccinia graminis,* but it is likely that a heterozygous condition also exists commonly in physiologic races of other cereal rusts. In *Puccinia anomala,* d'Oliveira (155) has demonstrated the heterozygosity of three physiologic races, one of which, when selfed, produced 6 different races. Similarly Murphy (138) proved the heterozygosity of race 2 of *Puccinia coronata Avenae* by infecting *Rhamnus* spp. with it and re-isolating six physiologic races from the aecia.

Crossing studies with physiologic races of Puccinia graminis

Inheritance of urediospore color. Almost all samples of stem rust collected in nature have red urediospores, and most samples are homozygous for this character. Occasional samples of stem rust, however, produce, when selfed, strains with urediospores of atypical color (72, 143). It is the discovery of such strains of rust that has made possible a study of the inheritance of urediospore color.

Crosses between two physiologic races of *P. graminis Tritici* with urediospores of atypical color have been reported (103, 153). One of these races had orange urediospores with bright yellow or orange pigment in the cytoplasm but no dark pigment in the spore wall.

The other race had greyish brown urediospores that showed little if any orange pigment in the cytoplasm but contained a pigmented spore wall. Rust of the normal (red) color contains both pigments. Crosses between the two races of atypical color produced rust with urediospores of the normal red color. Selfing of the hybrid rust produced a progeny with urediospores of four distinct colors—red, orange, greyish-brown and white. The size of the color classes suggested a 9 : 3 : 3 : 1 ratio. According to this ratio, the inheritance of urediospore color could be explained by assuming the presence of two pairs of color factors, GG governing pigmentation of the spore wall and YY governing that of the cytoplasm, both of which are present in spores of normal red color. The genetic constitution of the greyish-brown spores in which cytoplasmic pigment was absent would then be GG yy; and that of the orange spores lacking the spore-wall pigment would be gg YY. The association of the two gametes in F_1 would produce the constitution GgYy which would account for the ratio 9 red, 3 orange, 3 greyish-brown and 1 white. The white spores, thus encountered in stem rust for the first time, appeared to lack pigmentation in either spore wall or cytoplasm, and presumably possessed the constitution ggyy.

The urediospores of the atypical strains were not the only spore-forms of these strains showing differences in color from normal rust. Greyish-brown and white strains produced aecia that were pale yellow or almost white in appearance with aeciospores lacking orange pigments in the cytoplasm. Teliospores of greyish-brown strains resembled those of red strains, owing to the presence of dark pigment in the spore walls, but teliospores of the orange and the white strains were distinctly paler than normal, owing to deficiency of the spore-wall pigment.

Although in the cross discussed above there is evidence for a simple two-factor scheme of color inheritance, it does not necessarily follow that the same two pairs of factors apply to all color inheritance in stem rust. The existence of minute variation in the color of normal, red rust collected in nature (55, 234), and the occasional occurrence, in hybridization work, of different shades of orange or of greyish-brown color, would suggest the existence of a number of alleles responsible for urediospore color.

Apart from the crosses described above, the only other crosses between strains of rust differing in color that have thus far been

reported are those between races of *P. graminis Avenae* with red and orange urediospores (99). In F_1, red color proved dominant to orange. Information not yet published suggests a distribution of 3 red to 1 orange in the F_2 generation.

Inheritance of pathogenic characters. As indicated in the above section on the selfing of physiologic races, it is necessary, if intelligible results on the genetics of pathogenicity are to be obtained, to deal at any one time with a pair of contrasted pathogenic characters on a given host variety. In the cross race 9 × race 36 of *P. graminis Tritici* (98, 153), the contrasted characters of which the inheritance may be studied are given below together with the infection types produced by the hybrid rust:

		Infection types on host varieties				
		Kanred	Arnautka	Mindum	Spelmars	Vernal
(Parents)	Race 9	0	4	4	4	4
	Race 36	4	1	1	1	0
(Hybrid rust)	Race 17	0	4	4	4	0

If one considers first the contrasted pair of characters on the variety Kanred, the question to be decided by means of the cross is what infection type the hybrid rust produces on this variety. Each time the cross was made it was found that the hybrid rust was unable to rust Kanred, *i.e.,* the infection type was 0. The 0 type was, therefore, obviously dominant to the 4 type. On the varieties Arnautka, Mindum and Spelmars, the hybrid rust produced large sori (4 type), which showed that the 4 type was dominant to the 1 type (small sori). On the variety Vernal it was found that the hybrid rust produced a 0 type of infection. The 0 type produced on Vernal by race 36 was consequently dominant to the 4 type produced by race 9. Further work (98) has shown dominance and recessiveness of the same characters in crosses between other races, a fact indicating that these results are not peculiar to crosses between these two races but are of general, though not necessarily universal, occurrence in crosses between races of wheat stem rust.

Selfing studies with the F_1 hybrid rust (race 17) have shown that on the variety Kanred the 0 type, which was dominant in F_1, reappears in F_2 about three times as often as the recessive 4 type, thus indicating that a single pair of factors is operative. Similarly, on the durum varieties Arnautka, Mindum and Spelmars, the dominant 4 type occurs in F_2 about three times as frequently as the

recessive 1 type, suggesting again a single pair of alleles. With respect to the variety Vernal, however, a different situation exists. In F_2, the dominant character, the 0 type, appeared about 18 times as frequently as the contrasting recessive character, a distribution suggesting that at least two pairs of factors are operative. As the factors governing these characters appeared to segregate and recombine at random, it was possible for several physiologic races to appear in the F_2 generation. On the assumption that the pathogenic behaviour of the rust is governed by one-factor pairs for Kanred and the durums Arnautka, Mindum and Spelmars, and by a two-factor pair for Vernal, it was expected that the F_2 generation would be composed of races 1, 9, 11, 15, 17, 36, 52 and 57. Actually all these races did occur in the F_2 generation and comprised the bulk of it. Four additional races, however, appeared, namely, races 29, 32, 85 and 110. As each of these four races resembles one or another of those in the first-mentioned group, they were regarded as merely variants and grouped with the races they resembled, *i.e.*, race 29 was considered a variant of race 17, race 32 of race 11, race 85 of race 9, and race 110 of race 15. The actual and theoretical distributions in F_2 are shown below:

	Race	17	11	1	36	9	57	15	52
Distribution	Theor.	171.4	57.1	57.1	19.0	11.4	3.8	3.8	1.3
	Actual	165[a]	64[b]	47	31	11[c]	4	2[d]	1

[a] Includes 6 cultures of the related race 29.
[b] " 5 " " " " " 32.
[c] " 6 " " " " " 85.
[d] " 1 " " " " " 110.

This study shows that Mendelian laws are operative in the inheritance of the pathogenic characteristics of stem rust of wheat, and indicates, furthermore, that an understanding of the way in which these characteristics are inherited can lead to a considerable amount of prediction as to which physiologic races may be expected to arise from crosses between known races.

In stem rust of oats (*P. graminis Avenae*) it has been shown, likewise, that certain pathogenic characters are dominant to others (99), and it is indeed probable that the inheritance of pathogenicity follows much the same pattern as in wheat stem rust.

Cytoplasmic inheritance. The pathogenic characters discussed above, as already stated, appear to be inherited according to Mendelian laws. It does not follow, however, that all pathogenic traits

of stem rust are thus inherited. In certain crosses between physiologic races of wheat stem rust there is evidence that some pathogenic characters are inherited through the medium of the cytoplasm of the maternal parent race, *i.e.*, the race receiving a transfer of pycnial exudate. In crosses in which this type of inheritance is manifested, the pathogenic characteristics of the hybrid rusts arising from opposite sides of reciprocal crosses are not identical. Hence in the cross race 14 × race 36, the hybrid rust arising from the race –14 side of the cross conformed exactly to race 14, whereas the hybrid rust originating from the race –36 side of the cross was identified as race 88. The only difference between the reciprocal hybrid rusts was that, in the F_1 progeny from the race –14 side, the infection on Marquis was type 1 (as in the race –14 parent), whereas in the progeny arising from the reciprocal cross it was type X (as if influenced by the type 4 produced by the race –36 parent). The hybrid rusts arising from the reciprocal crosses were selfed to determine how far this cytoplasmic influence persisted in F_2 and F_3. The results (103, 144) showed a definite persistence in these generations of the infection types displayed on Marquis in F_1, a condition that would be expected if the original phenomenon were due to cytoplasmic influence.

A similar and even more clear-cut case of cytoplasmic inheritance has occurred in *P. graminis Avenae* in crosses between race 7 and race 11[8] (99). Race 7 produces on the variety Sevnothree a 4 type of infection (large sori), while race 11 produces a 1 type (small sori). The races arising from the race –7 side of a cross resemble race 7 in that they produce the 4 type on Sevnothree, while the races arising from the race –11 side resemble race 11 in that they produce the 1 type on this variety. This situation holds true not only for all crosses between race 7 and race 11 but apparently also for all crosses in which the two parent races differ in their infection types on Sevnothree. Consequently the same physiologic race never arises from a cross and its reciprocal cross.

This phenomenon does not seem capable of explanation on a purely chromosomal basis, but can be readily explained if it is assumed that the pycniospore nucleus of the paternal race reaches the protoaecium of the maternal race unaccompanied by its cytoplasm, which, in any case, is very limited on account of the minute

[8] Race 11 was originally numbered 10*a*.

size of the pycniospore. Each hybrid aeciospore would then receive from the paternal race only a nucleus, but from the maternal race a nucleus plus cytoplasm. This explanation, according to Lamb (110), "finds an entirely convincing cytological explanation" in his study of the initiation of the binucleate condition in *Puccinia Phragmitis* (Schum.) Körn.

In cereal rusts other than *P. graminis* only one record of possible cytoplasmic inheritance is known. D'Oliveira (155), in reciprocal crosses between races 13 and 23 of *Puccinia anomala,* obtained race 30 from the race –23 side of a cross but isolated two races, 30 and 18, from the reciprocal cross. Assuming that the infections were of monosporidial origin and that the nuclei of each haploid infection were identical, he attributes this result to differences in the cytoplasm among the hyphae that were diploidised.

Effect of inbreeding on rust characteristics. Each cereal rust possesses certain well defined characteristics of color, sporulation, *etc.,* which investigators regard as normal for the species; and, in rust collected in nature, deviation from a condition of normality is a rare occurrence. It was only when the component physiologic races of a rust came to be selfed—*i.e.,* when the alternate host was inoculated by a pure culture of a physiologic race followed by a close study of the uredial progeny arising from the aecia—that abnormal rust characteristics were observed in any considerable number. Though extremely rare in nature, deviation from the normal color of the uredia of *P. graminis* has been observed a number of times after the rust was induced to complete its life cycle by passage through its alternate host (72, 143, 196, 234).

Other abnormalities have also been noted, particularly when selected strains of *P. graminis Tritici* were selfed for several successive generations (97). The abnormalities observed were generally of a kind that under natural conditions would be detrimental to the survival of the rust, such as decrease in vigor of sporulation, *i.e.,* a tendency to form uredia with only a limited ability to rupture the epidermis, or decrease in pathogenic vigor as shown, for example, in the production of an X type of infection by cultures descended from strains that had appeared homozygous for the more vigorous 4 type of infection. Furthermore, certain strains originating through selfing have shown a greater sensitivity to high temperatures than strains collected in the field. Wheat varieties susceptible

to such strains at ordinary greenhouse temperatures develop resistance toward them at temperatures above 80° F. (96).

Unusual characteristics encountered in selfing studies are not limited to the uredial stage of the rust. Abnormalities of the pycnial and aecial stages have been noted on several occasions. Cotter (27) observed the production on barberry by *P. graminis Agrostidis* of white pycnia with colorless exudate. Intermixing the exudate of these pycnia led to the formation of white aecia, whereas application to white pycnia of exudate from the normal yellow pycnia resulted in the formation of yellow aecia, as did also the application of exudate from white to yellow pycnia. Evidently, white aecial color is recessive to yellow.

In *P. graminis Tritici* the selfing of certain physiologic races has brought about results similar to though not identical with those just mentioned. Newton and Johnson (146) observed that in the selfing of a culture of race 21 two types of pustules were produced in about equal numbers on leaves of barberry. Pustules of one type were normal in appearance and produced aecia following the intermixing of the exudate. Pustules of the other type were almost white with either no pycnia or only a few scattered ones that produced exudate only after a considerable period. Formation of aecia in white pustules was rare and nearly always limited to rudimentary or otherwise atypical structures. A small proportion of the white pustules produced urediospores and teliospores after a prolonged period. By selfing studies and by crosses with another physiologic race, it was shown (101) that diploidisation of the mycelia of normal pustules by pycniospores from white pustules gave rise to physiologic races that produced white and normal pustules on the barberry in approximately equal numbers, whereas normal x normal matings produced normal rust, and white x white matings were sterile. It was assumed that a mutation affecting one of the two conjugate nuclei took place in the original culture of race 21 and that during meiotic divisions in the germinating teliospore the mutant factor is segregated so that half of the sporidia give rise to white and half to normal pustules.

A somewhat different effect that appears to be attributable to inbreeding is the loss of ability to produce aecia in certain strains of *P. graminis Tritici* (97, 146). This peculiarity appeared in several F_3 cultures of a cross between races 9 and 36. The pycnia of these

cultures produced scanty exudate but formed no aecia as a result of either intermixing this exudate or application to them of the exudate of normal pycnia. In some of these cultures the loss of ability to produce aecia was associated with a rather marked tendency towards the production of urediospores and teliospores in old pycnial pustules. Some of these urediospores were found to be uninucleate (147).

The occurrence of uredia and telia of *Puccinia graminis* on barberry may possibly have some evolutionary significance in explaining the origin of short-cycled rusts from heteroecious long-cycled forms. Jackson (92) has postulated that microcyclic forms arise from the haploid generation of heteroecious eu-forms through the replacement of aecia by telia. Short-cycled species which appear to have thus originated are known as "correlated" species. The production of uredia and telia on barberry may perhaps be interpreted as evidence of a tendency on the part of *P. graminis* towards the formation of such a correlated species.

It is evident from the results given above that selfing of physiologic races, and particularly continued selfing of the same lines, brings to light characteristics unfavorable to the survival of the species. Such characteristics probably arise also under natural conditions but, not being adapted to survival, are soon eliminated so that they are infrequently encountered. In conclusion it may be stated that the development of abnormal strains of rust is not an inevitable consequence of inbreeding, as many inbred strains show no abnormalities. It is likely that abnormal characteristics are, in most cases, the result of recessive mutations that have taken place in the past history of the rust, the part played by selfing being that of segregating and recombining the mutant factors in a homozygous state under which condition their effects are manifested in various types of abnormalities.

Crosses between varieties of Puccinia graminis

The crossing studies already dealt with are concerned solely with intra-varietal crosses, *i.e.*, crosses between physiologic races of the same variety of stem rust. In such crosses interfertility of the different races appears to be complete. In inter-varietal crosses, on the other hand, some inter-sterility exists, the degree of which varies according to which varieties are crossed.

In crosses between the varieties *Tritici* and *Agrostidis,* Stakman *et al.* (196) found that when exudate from *Tritici* pycnia was transferred to *Agrostidis* pycnia no aecia were formed, but that when the transfer was made in the opposite direction aecia were formed in every case. Johnson *et al.* (102), in crosses between the same two varieties, transferred pycnial exudate from haploid *Agristidis* pustules to 14 haploid *Tritici* pustules, of which only one produced hybrid aecia. Transfers of exudate from *Tritici* pustules to 36 haploid *Agrostidis* pustules failed to produce any hybrid aecia. Crosses between the varieties *Tritici* and *Secalis,* however, led to the conclusion that little, if any, inter-sterility existed between these two varieties. In crosses between the varieties *Tritici* and *Avenae* (95) there was evidence of considerable intersterility.

One of the chief objects of making inter-varietal crosses is to discover the characteristic pathogenic properties of the hybrid rusts. Owing to several causes, this object has not always been successfully attained. In some crosses the great variety of pathogenic types occurring in the F_1 generation has made it difficult to interpret the results. In the crosses made by Stakman *et al.* (196) between *P. graminis Tritici* race 36 and *P. graminis Secalis* race 11, the F_1 progeny was resolved into eight physiologic races of *P. graminis Tritici,* namely, races 15, 21, 32, 36, 57, 67, 70 and 71, and two physiologic races of *P. graminis Secalis,* races 9 and 11. As far as can be judged from the pathogenicity of these races, only two of them (races 70 and 71 of *P. gr. Tritici*) could be considered at all intermediate between the *Tritici* and the *Secalis* varieties. The remainder of the races were either typical *Tritici* or typical *Secalis* races. These races may be hybrids in which *Tritici* or *Secalis* characters respectively predominate or, possibly, they may have resulted from accidental selfing of one or the other of the parental races. In crosses between the same two varieties, Johnson *et al.* (102) obtained an F_1 generation composed of four physiologic races identified as races 70, 104, 111 and 112 of *P. graminis Tritici.* Though described as races of wheat stem rust, they were remarkably weak in their pathogenicity on wheat varieties and even weaker in pathogenicity on rye. They lacked the ability to infect the wheat variety Acme which is attacked by nearly all other physiologic races of wheat stem-rust. Like both the parent varieties used in the crosses, they were capable of attacking barley. These four

races, though distinguishable, are pathogenically similar and form, together with race 71 (mentioned above), a well defined group with pathogenic characters that may be considered more or less intermediate between those of the *Tritici* and *Secalis* varieties of stem rust. Even more narrowly limited to barley are certain hybrids from crosses between the varieties *Tritici* and *Secalis* reported by Levine, Cotter and Stakman (117).

A somewhat different result of *Secalis-Tritici* crosses is reported by Levine and Cotter (116) who described a hybrid rust capable of attacking both wheat and rye rather severely as well as barley. This rust, therefore, combines to a large degree the pathogenic powers of both parental varieties.

It seems probable that these two types of hybrids—the one possessing only to a limited degree the pathogenic powers of the parents, the other possessing a combination of their pathogenic properties—are both authentic and perhaps representative F_1 hybrids. In view of the great diversity of pathogenic types found in the parental rusts it is not surprising that considerable variation should likewise be found in their hybrid progeny.

Interpretation of the results of crosses between the varieties *Agrostidis* and *Tritici* is confronted with much the same difficulties as those already outlined in connection with *Tritici-Secalis* crosses. In crosses reported by Stakman *et al.* (196) the progeny, all of which arose on the *Tritici* side of the crosses, was composed of races that attacked wheat but not *Agrostis* species. At present it cannot be definitely decided whether the races are actual hybrids or the result of fortuitous selfing.

A *Tritici-Agrostidis* cross reported by Johnson *et al.* (102) produced different results. The progeny, which arose on the *Tritici* side of the cross, possessed to a very limited degree the pathogenic properties of both parental rusts. It was able to infect both wheat and *Agrostis* but only to a very slight extent. Like the *Tritici* parent rust it was capable of infecting barley, though not heavily.

A cross between the varieties *Tritici* and *Avenae* has been reported by Johnson and Newton (95). The hybrid, which originated on the *Tritici* side of the cross, possessed some of the pathogenic properties of both parental rusts. From the *Tritici* parent it inherited the ability to attack, though rather lightly, certain wheat and barley varieties. From the *Avenae* parent it inherited the

capacity to attack certain varieties of oats and some of the grasses that are natural hosts of oat stem rust. The host range of the hybrid rust was, therefore, considerably wider than that of either parent rust; but this extension of host range was accomplished at the expense of pathogenic vigor, as the hybrid rust attacked any given host more lightly than did the parental rust of that host.

In general it would seem from the work thus far reported on inter-varietal hybridization in *Puccinia graminis* that hybrid rusts frequently show some of the pathogenic properties of both parental strains, but that this widening of host range is accompanied by a loss of pathogenic vigor towards any given host—a loss in some instances so great that the rust can find no really congenial host.

MUTATION

It is evident from the preceding discussion on hybridization that crossing and selfing of the different strains of a rust are capable of giving rise to much variation. The function of hybridization, however, is chiefly that of segregating and recombining the great variety of factors that already exist in the organism so that the greatest possible number of combinations may take place. Hybridization *per se* does not answer the question of how these factors came into being or how rapidly they change.

The stability of the genetic factors governing rust characteristics is a matter of interest because changes in these factors involve changes in the characteristics of the rust. If such changes are of frequent occurrence, it may be supposed that the adaptability of the rust to new conditions would be thereby increased.

During the relatively short period that has elapsed since cereal rusts came to be studied intensively, a considerable number of changes ascribable to mutation have been recorded. Changes affecting the color of a rust are perhaps the easiest to detect; and it is consequently not surprising that the first record of mutation in the cereal rusts should be of this type. Two deviations from the normal red color of urediospores of *P. graminis Tritici* were noted by Newton and Johnson (143)—one a change to orange, involving a loss of pigment in the spore wall, the other a change to grayish-brown, resulting from loss of yellow or orange pigment in the cytoplasm. The first occurred in a uredial culture under study in the greenhouse; the second was observed in uredia derived from

aecia on artificially inoculated barberry, and was later (152) found to occur in uredia derived from the selfing of a pure culture of race 36. A mutation involving the production of yellow uredia was also reported by Waterhouse (234) in a culture of race 34 of wheat stem rust. Cotter and Levine (28) observed on two separate occasions in uredial cultures of *P. graminis Secalis* race 7, yellow uredia that gave rise to yellow strains of this race. In *P. graminis Avenae* Gordon (72) discovered orange strains of two physiologic races in uredial cultures obtained from aecia on a barberry inoculated with teliospore material gathered in the field. As grayish-brown and orange spore color is recessive to red, the above-mentioned grayish-brown and orange strains originating from aecia may perhaps be considered to have arisen from mutations that had taken place in the rust at some previous time. Possibly the same explanation may account for the origin of the brown and yellow strains of wheat stem rust discovered by Stakman *et al.* (196) in the progeny of *Tritici-Agrostidis* crosses.

Though some slight variation exists in uredial color of collections of stem rust gathered in the field (143, 234), radical departures from normal color are very rarely met with in nature. One such, however, a grayish-brown collection of oat stem rust is reported by Newton and Johnson (150), and another is possibly represented by a collection of race 40 of wheat stem rust with ochre colored uredia reported by Garbowski (55). It seems likely that striking deviation from normal spore color occurs among uredia derived from naturally infected barberries, but that such abnormal strains are not well adapted for survival and are thus infrequently encountered. It is probable, indeed, that the normal pigmentation of stem rust urediospores is a good protective agent against the ultra-violet light produced by the sun, for it has been shown (37) that spores of orange and white strains of wheat stem rust are more readily destroyed by ultra-violet light than those of grayish-brown or normal color.

Two records are known of what appear to be mutations involving both uredial color and pathogenicity. Johnston (104) described a field collection of *Puccinia triticina* differing pathogenically from known races of this rust and characterized by a long incubation period and by uredia lighter in color than those of other races. D'Oliveira (155) reported the production, in race 14 of *Puccinia*

anomala, of an orange mutant differing pathogenically from the culture in which it appeared. Subsequent work showed at least one recurrence of the same mutation.

A number of mutations for pathogenic characters have been recorded in the cereal rusts. In *P. graminis Tritici,* mutations affecting pathogenicity have been described by Stakman, Levine and Cotter (196) who observed four separate mutations in the same physiologic race; and by Newton and Johnson (148) who reported an instance of a known physiologic race being gradually replaced by a previously unknown race. In *P. graminis Avenae* the last mentioned authors (150) encountered a somewhat similar case of the displacement of one physiologic race by another through a recurrence of the same mutation. In *P. triticina* Roberts (175) reported a mutation for pathogenicity in a culture under study in the greenhouse; and in *P. glumarum* Gassner and Straib (66) described a series of recurrent mutations resulting in identical mutants of greater infectivity than the original race from which they arose.

It will be evident from the instances cited above that mutation in cereal rusts cannot be regarded as an extremely rare phenomenon. Because it is usually difficult to prove that a mutation for pathogenicity has occurred, it is likely that many more such mutations have occurred than have been reported. Although mutations are not rare occurrences, it is probably not justifiable to conclude that isolated mutations occur at random in the various strains of a rust. It seems more likely that certain strains now and then develop a condition of instability which leads either to the production of several different mutations, as in the case reported by Stakman *et al.* (196), or, as seems to have happened more frequently, to the recurrence of the same mutations (28, 66, 148, 150, 155). The great majority of rust strains that have been kept in culture over any considerable period have given no evidence of mutability.

Not all mutations that take place in a rust are immediately observable. Selfing studies, in particular, have shown that there may be latent in a normal rust certain abnormal characteristics that come to light only when the rust is selfed. These are governed by recessive factors that presumably originate by mutation and become observable only when they are present in the homozygous condition. Although abnormal characteristics, such as those of color, are not

well fitted for survival, it does not follow that all such unexpressed mutations cannot survive when brought to light. There exist in many strains of rust latent pathogenic potentialities that may reach expression in the new rust generation developing from the infected alternate host. These potentialities, perhaps originating in some genic changes of the past, may thus become actualities and possibly permanent rust characteristics. There can be no question that *Berberis vulgaris,* the alternate host of *Puccinia graminis,* plays its part in giving expression to the most diverse pathogenic characteristics. Extensive studies by Stakman *et al.* (197) showed that in aecial collections of *P. graminis Tritici* a different race was identified for every 4 collections, whereas in uredial collections made at random a different race was identified for every 100 collections. Even if only a fraction of the immensely greater variation evident in the rust derived from the barberry is originally due to mutation, there would still be ample reason to consider mutation a factor of great importance.

CONCLUSION

The 50 years that have elapsed since Eriksson's discovery of physiologic specialization in the cereal rusts have seen notable advances in man's understanding of these important pathogens. The discovery that the *formae speciales* or varieties, which were at first regarded as the ultimate units of specialization, were in turn made up of many specialized strains or physiologic races gave a great impetus to the study of the various rusts and, despite the fact that the existence of these physiologic races complicated the problem of developing rust resistant varieties of cereals, served also as a stimulus to greater efforts on the part of the breeders of rust resistant cereal varieties. The discovery of the existence of heterothallism in the rusts made possible studies on the crossing and selfing of the physiologic races of cereal rusts. Such studies have indicated that a great variety of physiologic races may be expected to arise by hybridization and have permitted a much more accurate evaluation than otherwise would have been possible of the significance of the alternate hosts in the production of physiologic races. These studies have also thrown considerable light on the genetics of the rust fungi and have shown that the inheritance of such rust characteristics as pathogenicity and spore color is subject to the

same laws that have been found to govern inheritance in higher plants and animals.

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